Effect of Certain Immunomodulators on Uterine Infections and Fertility in Post Partum Buffaloes

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ABSTRACT : The present study was aimed to study the effect of aqueous extract of *Tinospora cordifolia* and autologous plasma on uterine infections. Buffaloes in estrus, 40 days and above postpartum were checked for uterine infections. Animals having uterine infections were randomly divided into three groups of six animals each. Buffaloes in group I, II and III (control) were given intrauterine infusion of aqueous extract of Tinospora cordifolia (3,000 mg total dose), autologous plasma (150 ml) and phosphate buffer saline (150 ml) respectively, in three divided doses, once daily for three consecutive days, starting from the day of estrus. A fourth group (IV) comprising of six buffaloes having no uterine infection was also included in the present study. Buffaloes were inseminated artificially on next estrus following treatment and confirmed for pregnancy 60 days later. Bacterial population in CVM of buffaloes in group I, II and III was significantly ($p<0.05$) higher than group IV. After treatment there was a significant ($p<0.01$) reduction in bacterial population in group I (83.496±7.755%) and group II (80.233±5.799%) than group in III (7.557±33.551%) at next estrus. There was non-significant improvement in first service conception rate (CR, 33.33%) and overall conception rate (OCR, 27.27%) in group I, in comparison to group III (first service CR-16.67%; OCR-20.0%). No significant improvement was seen in OCR (22.22%) in-group II also in comparison to group III. The improvement in group I was however, nonsignificantly lower than normal animals of group IV (First service CR-16.67%; OCR-36.33%). ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 7 : 930-935)***

Key Words : Buffaloes, Uterine Infection *Tinospora cordifolia*, Autologous Plasma, Bacterial Load, Conception Rate

INTRODUCTION

Buffaloes form a important part of livestock resources of tropical countries and contribute in milk and meat production substantially.

Disease of any system has adverse effect on reproduction and ultimately lowers calf crop and milk production. Of all the diseases of reproductive system, incidence of uterine infection is more and that too under field conditions, due to lowered immune status and unhygienic conditions prevailing during parturition. Difficulty in controlling predisposing factors under field conditions has drawn more attention towards the therapeutic management of the diseases. Various substances, ranging from antibiotics to hormones have been tried earlier to counteract uterine infections. But these conventional therapies besides being costly causes extra harm to animal in form of development of resistance for them in microbes, residues in animal products, diseases like cyst formation etc.

Use of certain immunomodulatory substances, as alternative therapeutic agents have become a subject of recent scientific investigations. *Tinospora cordifolia*, an indigenous plant used in Ayurveda is known for its immunopotentiating action, and has been shown to have beneficial effect in burns, ulcer, cancer etc. and mastitis in cattle (Singh, 2000). Similarly plasma has been shown to have local inflammatory effect on endometrium in mares (Waelchli et al., 1987) in addition to the bactericidal and bacteriolytic activity (Myrvik, 1956; Taylor, 1987). Autologous or homologous plasma has been used in cattle (Venugopal, 1985; Saini et al., 1999) and mares (Asbury, 1984; Ward, 1985) with varying success. No such studies have been carried out in buffaloes.

Hence, the present experiment was conducted to test the efficacy of autologous plasma and extract of *Tinospora cordifolia* on uterine infections and effect of the above therapies on subsequent conception in buffaloes.

MATERIALS AND METHODS

The study was carried out on Murrah buffaloes (n=24) maintained at Livestock production and management farm, Indian Veterinary Research Institute, Izatnagar, India. All the animals were maintained in one- fourth covered shed under loose housing system. They were fed with balanced ration and given free supply of drinking water. All the buffaloes were in age group of 3-7 years and in their 2nd to 4th lactation.

Buffaloes having uterine infection were selected on the basis of history of repeat breeding, per rectal examination of the genitalia, bacterial load in cervico-vaginal mucous (CVM), any abnormality (pus, flakes, blood etc.) in CVM, pH and color reaction of CVM to white side test (Popov, 1969). CVM with abnormal color, consistency, high alkaline pH, high bacterial load and positive white side test was considered as an indicator of uterine infection. The presence of uterine infection was confirmed by bacteriological culture and identification of organisms in

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Table 1. Mean (±SE) pH values and intensity of color reaction of crevico-vaginal mucus to White side test (WST) in Buffaloes treated with different immunomodulatory substances

Group	Pre-Treatment WST - pH									Post-Treatment WST - pH								
	D	pH	M	pH	L	pH	N	pH	Overall pH	D	pH	M	pH	L	pH	N	pH	Overall pH
I N=6	1 (16.67%)	8.80±0.02	2 (33.33%)	8.70±0.01	3 (50.00%)	8.30±0.10	0	-	8.52^{A}±0.11	0	-	2 (33.33%)	8.50±0.00	0	-	4 (66.67%)	7.90±0.10	8.10^{a}±0.14
II N=6	1 (16.67%)	8.80±0.00	4 (66.67%)	8.72±0.15	1 (16.67%)	8.20±0.00	0	-	8.63^{Aa}±0.02	0	-	0		2 (33.33%)	8.15±0.05	4 (66.6 %)	7.65±0.15	7.828^{b}±0.19
III N=6	2 (33.33%)	8.87±0.03	2 (33.33%)	8.60±0.00	2 (33.33%)	8.10±0.20	0	-	8.57^{A}±0.16	0	-	2 (33.33%)	8.75±0.25	2 (33.33%)	8.20±0.00	2 (33.33%)	7.80±0.20	8.25^{a}±0.19
IV N=6	0	-	0	-	0	-	6 (100 %)	-	7.70^{b}±0.12	0	-	0		0	-	6 (100 %)	7.70±0.12	7.70^{b}±0.12

Values within same rows with different superscript (A. B) differ significantly ($p<0.05$) and within same column with different superscript (a. b) differ significantly ($p<0.01$). D: Dark, M: Moderate, L: Light, N: No.

Table 2. Effect of different treatments on bacterial load in cervico- vaginal mucus of buffalo

Group	Bacterial Load ($\times10^4$/ml) in CVM		Percent (%) reduction
	Pre-treatment estrus	Post-treatment estrus	
I (*Tinospora cordifolia*)	209.125±43.016 ^{Aa}	32.083±18.412 ^{Ba}	83.496±7.755
II (Autologous plasma)	204.848±36.336 ^{Aa}	44.230±18.833 ^{Bac}	80.233±5.799
III* (Phosphate buffer saline)	154.367±13.943 ^{a}	137.790±36.178 ^{b}	7.557±33.551
IV (Normal)	96.218±10.354 ^{b}	96.218±10.354 ^{bc}	

All values with different superscript ($^{A, B}$) within same row and with different superscript ($^{a, b, c}$) within same column differ significantly at $p<0.01$ and $p<0.05$ respectively. * Control.

uterine secretions as per methodology described by Cowan (1974).

Buffaloes having uterine infection were divided into three groups of six animals each. Buffaloes in group I were given intrauterine infusion of 50 ml of sterile aqueous extract of *Tinospora cordifolia* (20 mg/ml) (Immunod tablet, M/S Merind Ltd.) daily for three consecutive days, starting on day of estrus. Buffaloes in group II were given 50 ml of autologous plasma intrauterine and buffaloes in group III were given 50 ml of sterile phosphate buffered saline (PBS) intrauterine, for three consecutive days. Autologous plasma was obtained from blood collected from buffaloes in estrus, stored at -20°C and used in the same buffalo. Care was taken to maintain sterility of intrauterine infusions. A fourth group comprising of six normal buffaloes was also included in the study with no treatment being given to them.

The buffaloes were examined for the presence of uterine infection on estrus prior to treatment and on subsequent estrus following treatment. The efficacy of therapy was judged by negative white side test, neutral or nearly neutral pH of CVM, reduced bacterial load and negative bacterial culture in the CVM.

Buffaloes in estrus were detected by parading vasectomized bull for 30-40 minutes in the shed, twice daily, in morning and evening. Buffaloes in group I, II and III were inseminated on subsequent estrus (estrus following treatment) and buffaloes in group IV on same estrus, using 0.25 ml frozen semen straws from a single fertile bull. Pregnancy was confirmed per rectally 60 days post insemination. Non-pregnant animals were re inseminated.

Statistical analysis of data was done using paired t-test and Duncan multiple range test (D.M.R.T.) (Snedecor and Cochran, 1989).

RESULTS

All the buffaloes in group I, II and III were tested positive for white side test prior to treatment. After treatment only 33.33% buffaloes in group I, 33.33% in group II and 66.67% buffaloes in group III gave positive color reactions. Buffaloes in group IV were negative for white side test.

Overall pH of CVM before treatment was 8.52±0.11 in group I, 8.63±0.02 in group II, 8.57±0.16 in group III and 7.70±0.12 in group IV. After treatment pH of CVM reduced significantly ($p<0.05$) in group II (7.82±0.19) and non-significantly in group I (8.10±0.14) and group III (8.25±0.19) (Table 1).

The bacterial load before treatment was significantly ($p<0.05$) higher in-group I (209.125±43.016), group II (204.848±36.336) and group III (154.367±13.943) than group IV (96.218±10.354) (Table 2). However, after treatment bacterial load in CVM was significantly ($p<0.01$) reduced by 83.496±7.755% in-group I and 80.233±5.799% in-group II. These post treatments bacterial load of CVM was significantly ($p<0.05$) lower in group I (32.083 ±18.412) and non-significantly lower in-group II (44.230± 18.833) than normal animals of group IV. In-group III there was a non-significant reduction of 7.557±33.55% in

Table 3. Bacterial isolates of cervico-vaginal mucus in treated and control buffaloes

Group	S. No. of Animal	Type of organism		Status
		Pre-treatment	Post-treatment	
I	1	*Alcaligens faecalis, Listeria monocytogenes.*	*Alcaligens faecalis, Listeria monocytogenes*	NP
(Tinospora cordifolia)	2	*Bacillus cereus, Staphylococcus aureus, Pseudomonas spp.*	*Bacillus cereus*	NP
	3	*Streptococcus spp., Listeria monocytogenes, Staphylococcus spp.*	*Streptococcus spp., Listeria monocytogenes, Staphylococcus spp*	NP
	4	*Bacillus cereus, Ecoli*	*Bacillus cereus, Ecoli*	P
	5	*Bacillus cereus, Staphylococcus aureus, Alcaligens faecalis*	*Not infected*	P
	6	*Alcaligens faecalis*	*Not infected*	P
II	1	*Bacillus spp., Staphylococcus aureus, Alkrococcusluteus*	*Staphylococcus aureus, Alicrococcus lute us, Neisseria spp.*	NP
(Autologous plasma)	2	*Corynebacterium spp., Alcaligens faecalis,*	*Not infected.*	P
	3	*Staphylococcus spp., Ecoli, Corynebacterium spp.*	*Ecoli, Staphylococcus spp.*	NP
	4	*Staphylococcus aureus, Ecoli*	*Staphylococcus aureus*	NP
	5	*Pseudomonas spp., Alcaligens faecalis, Ecoli*	*Alcaligens faecalis*	P
	6	*Staphylococcus aureus, Ecoli, Alcaligens faecalis, Listeria monocytogenes*	*Ecoli*	NP
III	1	*Pseudomonas spp., Ecoli, Alicrococcus lute us, Streptococcus spp.*	*Ecoli, Streptococcus spp.*	P
(Control)	2	*Pseudomonas spp., Ecoli*	*E coli,*	P
	3	*Alicrococcus luteus, Alcaligens faecalis, Corynebacterium spp*	*Alicrococcus luteus, Corynebacterium spp*	NP
	4	*Alcaligens faecalis*	*Alcaligens faecalis Pseudomonas spp.,*	NP
	5	*Bacillus cereus, Alicrococcus roseus*	*Bacillus cereus, Alicrococcus roseus*	NP
	6	*Staphylococcus aureus, Corynebacterium spp*	*Staphylococcus aureus*	NP
IV	1	*Alcaligens faecalis, Alicrococcus roseus*		NP
(Normal)	2	*Alicrococcus roseus, E coli,*		P
	3	*Alicrococcus luteus, Pseudomonas spp.*		NP
	4	*Alicrococcus luteus, Ecoli, Bacillus cereus*		P
	5	*Alicrococcus roseus*		P
	6	*Ecoli, Bacillus cereus*		P

P: pregnant, NP: non pregnant.

bacterial load to 137.790±36.178.

The type of organisms isolated before and after treatment, and their proportionate population is presented in Table 3 and 4 respectively. Majority (79.49%) of samples showed mixed type of infection, whereas some single isolates (20.51%) were also obtained. The different types of isolates obtained in decreasing level of frequency were *E. coli* (17.86%) *Alcaligens faecalis* (14.29%), *Bacillus cereus* (11.90%) *Staphylococcus aureus* (10.71%), *Pseudomonas spp.* (8.33%), *Micrococcus luteus* (8.33%), *Corynebacterium spp.* (5.95%), *Listeria monocytogenes* (5.95%), *Micrococcus roseus* (5.95%), *Staphylococcus spp.* (4.76%), *Streptococcus spp.* (4.76%) and *Neisseria spp.* (1.19%).

The data presented in Table 5 represents conception rate in different groups after treatment. The first service conception rate as well as overall conception rate in buffaloes treated with aqueous extract of *Tinospora cordifolia* (group I) was 33.33% and 27.27% respectively. In group II (treated with autologous plasma) the first service conception rate was 16.67%, whereas overall conception rate (OCR) was 22.22%. Similarly in group III first service conception rate and OCR was 16.67 and 20.0% respectively. In group IV, the CR at first service was similar (16.67%) to group II and III, but OCR was slightly higher (36.33%).

DISCUSSION

Bacterial load has been used as a diagnostic indicator for healthy status of an organ, including uterus (Dhaliwal et al., 2001). Significantly high bacterial load in CVM as observed in present study might be due to contamination of reproductive tract and failure of normal uterine defence mechanisms (UDM) to eliminate this infection. This could be either due to the suppression of UDM (Tarpstra et al., 1951) or the magnitude of infection is too large to cope with

Table 4. Proportionate population of organisms isolated from cervico-vaginal mucous of buffaloes with uterine infection

Type of bacteria	Number of isolates	Percent total
Alcaligens faecalis	12	14.29
Bacillus cereus	10	11.90
Corynebacterium spp.	5	5.95
E. coli	15	17.86
Listeria monocytogenes	5	5.95
Micrococcus luteus	7	8.33
Micrococcus roseus	5	5.95
Neisseria spp.	1	1.19
Pseudomonas spp.	7	8.33
Staphylococcus aureus	9	10.71
Staphylococcus spp.	4	4.76
Streptococcus spp.	4	4.76
Single	8	20.51
Mixed	31	79.49
Total isolates	84	100%

the normal UDM. The high bacterial population in present study indicates weak UDM, as most of the organisms identified are opportunistic pathogens. These organisms (Table 3) are normally found in posterior G.I. tract, around perineal area and in reproductive tract (Dhaliwal et al., 2001) and become pathogenic when their population flares up due to weak UDM. This may be the reason for their presence even in buffaloes giving no sign of infection (group IV). Several workers have reported similar organisms in cases of metritis in buffaloes (Pateria et al., 1989; Khan et al., 1990; Nguyen Van Thanh, 1997; Adhau et al., 1999; Arora et al., 2000 and Sharda and Krishnan, 2000). However, some organisms like *Listeria spp. Pseudomonas spp.* have been found to be pathogenic in animals (Roberts, 1986).

The increased number of bacteria leads to increased amount of bacterial metabolites, which damages the endometrium (Boiter et al., 1980). This causes increased pH of uterine fluid and CVM. This may be the possible reason for the high pH of CVM in buffaloes before treatment, in the present study. Our findings are in accordance with Boiter et al. (1980) who reported higher pH of CVM in cows suffering from endometritis.

The high bacterial population stimulates normal UDM. Presence of infection in uterus induces inflammatory condition and thereby leukocytosis occurs. This leukocytosis might be causing the development of color reaction to white side test. The appearance of color reaction has been positively co-related with the presence of inflammation by Popov (1969).

There was a significant ($p<0.01$) reduction in bacterial load of CVM in buffaloes treated with aqueous extract of *Tinospora cordifolia* (group I) and autologous plasma (group II). The bacterial load decreased by 83.496±7.755% in group I and 80.233±5.799% in group II. This reduction

Table 5. Fertility response in buffaloes treated with different immunomodulatory substances

Treatment group	No. of animals	Conception rate at first AI	Overall conception rate
I *(Tinospora cordifolia)*	6	33.33%	27.27%
II (Autologous plasma)	6	16.67%	22.22%
III* (Phosphate buffer saline)	6	16.67%	20.00%
IV** (Normal)	6	16.67%	36.33%

* control, ** non infected.

suggests the possible antibacterial effect of both the substances. *Tinospora cordifolia* has been suggested to have direct antibacterial effect (Direkbusarakom et al., 1988) besides causing non specific stimulation of immune response (Rege et al., 1999). It induces leukocytosis (Thatte et al., 1989) and activates macrophages (Prince and Menon, 1999). This may be the reason for white pus like mucoid discharge from the genital tract on the subsequent days following treatment. Rege and Dahanukar (1997) reported that it increases the phagocytic and killing capacity of peritoneal macrophages in rats and man in a dose dependant manner. Further, *Tinospora cordifolia* enhances antibody production by proliferation of B-cells (Sainis et al., 1997). The reduction in bacterial population in the present study may be due to this immunostimulatory activity of *T. cordifolia*. The activated phagocytic cells are more competent in phagocytizing invading bacteria. While the raised antibody level help in better opsonization of bacteria for better phagocytosis or, cause direct lysis of bacteria. Similarly, Singh (2000) reported reduction in bacterial load of milk samples in cases of mastitis in cows treated with intramammary infusion of aqueous extract of *T. cordifolia*.

The reduced bacterial load in CVM of buffaloes treated with autologous plasma might be due to the direct bactericidal and bacteriolytic effect of plasma (Myrvik, 1980; Taylor, 1987). Waelchli et al. (1987) reported that uterine culture became negative in most endometritic mares after seven days of intrauterine infusion of mare's own plasma. It is possible that substances such as complements and Immunoglobulins (Asbury, 1980) supplied by plasma help in better opsonization of bacteria and hence better phagocytosis (Asbury, 1984). Besides, various other proteins present in plasma, like Lysozyme (Feingold et al., 1968) might be helpful in killing bacteria. The chemoattractant present in plasma (Waelchli et al., 1987) enhance influx of neutrophils and hence better phagocytosis resulting in decreased bacterial load.

The non significant reduction (7.557±33.551%) in bacterial load in group II may be due to the local UDM or

the inflammatory effect produced by PBS (Waelchli et al., 1987).

The reduced bacterial population leads to reduced bacterial metabolites and hence reduced pH to near neutral level. Though change was significant only in group II, the final pH was non-significantly different in both the groups. Similarly leukocyte population is also reduced in absence of any infection and hence reduced percentage of positive color reaction to white side test (33.33% in group I, 33.33% in group II and 66.67% in group III).

Conception rates after artificial insemination on subsequent estrus didn't show any significant difference. The aqueous extract of *T. cordifolia* had slightly better results than autologous plasma (group II) and control group III. *T. cordifolia* did show significantly better results in terms of microbiological picture and other tests, than autologous plasma and PBS. It indicates a possible ameliorative effect of *T. cordifolia* on uterine infections. This together with its convenient availability than plasma can be considered as an alternative therapy in uterine infections.

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